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Ethanol inhibition of adenosine 5′-triphosphate-activated current in freshly isolated adult rat hippocampal CA1 neurons

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Abstract

The effect of ethanol on current activated by extracellular adenosine 5′-triphosphate (ATP) was studied in freshly isolated adult rat hippocampal CA1 neurons using whole-cell patch-clamp recording. ATP activated an inward current with an EC_{50} value of 18 μM. The inward current was also activated by 2-methylthio ATP (2-MeSATP) and α,β-methylene ATP (α,β-MeATP), inhibited by pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), and potentiated by Zn^{2+}. Ethanol inhibited current activated by 10 μM ATP with an IC_{50} value of 83 mM in a voltage-independent manner. Ethanol, 100 mM, shifted the ATP concentration-response curve to the right, increasing the EC_{50} value for ATP from 18 to 33 μM, but did not reduce the maximal response to ATP. The results suggest that ethanol can inhibit the function of P2X receptors in adult rat hippocampal neurons by decreasing the apparent affinity of the binding site for ATP.

Keywords: Adenosine 5′-triphosphate; Ion channels; P2X receptors; Hippocampus; Alcohol

P2X receptors are ligand-gated cation channels activated by extracellular adenosine 5′-triphosphate (ATP). These receptor-channels produce excitatory actions in both the central and peripheral nervous systems. For instance, activation of P2X receptors mediates excitatory synaptic transmission in guinea-pig autonomic ganglion neurons [6,8,21], rat spinal cord [2], and in the hippocampus [19], medial habenula [5] and locus coeruleus of rat brain [17]. To date, seven P2X receptor subunits, designated $P2X_1$–$P2X_7$, have been cloned and these subunits have been found to be widely distributed in the nervous system, including the cerebral cortex, hippocampus, thalamus, hypothalamus, midbrain, cerebellum and spinal cord in the central nervous system, and in sensory and autonomic ganglia in the peripheral nervous system [3,13,20,22]. Recent studies have revealed that P2X receptors in bullfrog dorsal root ganglion (DRG) neurons [14,23] and recombinant rat $P2X_4$ receptors expressed in *Xenopus* oocytes [27] are inhibited by pharmacological concentrations of ethanol. However, effects of ethanol on P2X receptors in the mammalian central nervous system have not been reported. In the present study, we investigated the effect of ethanol on current activated by extracellular ATP in neurons freshly isolated from the CA1 region of adult rat hippocampus.

The procedure for acutely dissociating neurons from the hippocampus was adapted from Kay and Wong [11], and whole-cell patch-clamp recording from visually identified pyramidal-shaped CA1 neurons was performed as described previously [14]. Briefly, adult male Sprague–Dawley rats (4–5 weeks) were decapitated and tissue chunks from the hippocampal CA1 region were dissected and digested in oxygenated piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)-buffered saline containing trypsin XI (0.5 mg/ml, Sigma) at 32°C for 60 min. CA1 neurons were isolated by trituration in 1 ml Dulbecco's modified Eagle's medium (DMEM, Gibco) with 25 mM HEPES. Neurons were plated in 35 × 10 mm uncoated plastic petri dishes and used for electrophysiological recording after at least 1 h, to allow for cell attachment. Animal care and use in this study was approved by the NIAAA Animal Care and Use Committee (Protocol LMCN-FW-01) in accordance with NIH guidelines. Membrane potential was held at −60 mV, except where indicated. Drug solutions were delivered from a linear barrel array as described previously [14]. Concentration-response curves were analyzed using the program

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ALLFIT [4] and statistical comparisons were performed using Student's *t*-test or analysis of variance (ANOVA), as noted. Average values are reported as mean peak amplitude ± SE of the mean.

Fig. 1 illustrates analysis of ATP-activated current in voltage-clamped freshly isolated rat hippocampal CA1 neurons. ATP application rapidly activated an inward current that showed desensitization at higher agonist concentrations and decayed quickly upon removal of ATP (Fig. 1A). Responses to ATP application were observed in 31% of the neurons studied (28–90 cells). The amplitude of the response was concentration-dependent; the EC_{50} value of the concentration-response curve for ATP was 18 ± 0.7 μM and the slope factor was 1.3 ± 0.1 μM (Fig. 1B). The P2 receptor agonists 2-methylthio ATP (2-MeSATP) and α,β-methylene ATP (α,β-MeATP) also activated inward current in these neurons (Fig. 1C, left traces; $n = 4$). In addition, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), a P2 receptor antagonist, inhibited ATP-activated current (Fig. 1C, right traces; $n = 4$). By contrast, the trace element Zn^{2+} potentiated the current activated by ATP (Fig. 1C, right traces; $n = 4$).

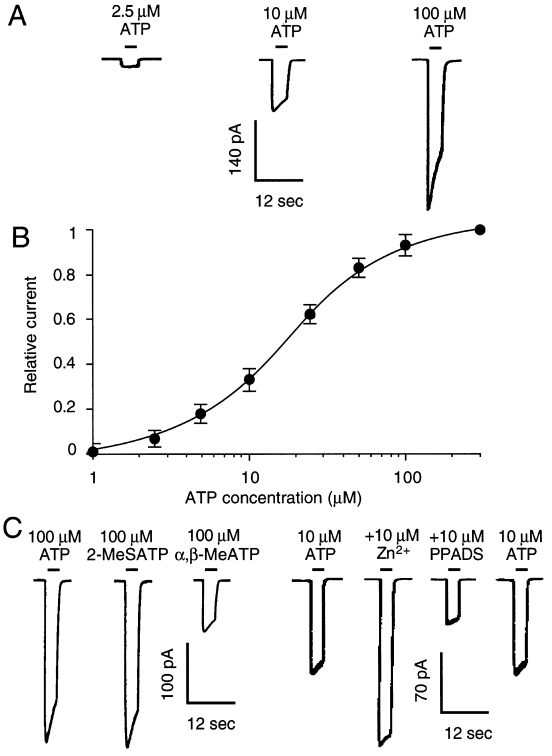

Fig. 1. ATP-activated inward current in freshly isolated adult rat hippocampal CA1 neurons. (A) Inward currents activated by 2.5, 10 and 100 μM ATP. Records are sequential current traces (from left to right) obtained from a single neuron. (B) Graph plotting the relative amplitude of ATP-activated current as a function of ATP concentration (1, 2.5, 5, 10, 25, 50, 100 and 300 μM). Amplitudes of currents activated by various concentrations of ATP were normalized to the current evoked by 300 μM ATP. Each point is the average of 4-5 cells; error bars indicate mean ± SE. The EC_{50} value of ATP was 18 ± 0.7 μM and the slope factor was 1.3 ± 0.1. (C) (Left traces) Ion current activated by 100 μM ATP, 100 μM 2-MeSATP and 100 μM α,β-MeATP in a single neuron. (Right traces) Ion current activated by 10 μM ATP in the absence and presence of 10 μM Zn^{2+} or 10 μM PPADS in another neuron.

Fig. 2 illustrates the effect of ethanol on ATP-activated current in hippocampal CA1 neurons. As shown in Fig. 2A, the current activated by 10 μM ATP was decreased in amplitude by 50 and 100 mM ethanol and recovered after ethanol washout, and 100 mM ethanol did not activate current in the absence of ATP. On average, 50 and 100 mM ethanol decreased the amplitude of current activated by 10 μM ATP by 40 ± 7% ($n = 8$) and 60 ± 9% ($n = 6$), respectively. In addition, 100 mM ethanol inhibited currents activated by 20 μM 2-MeSATP and α,β-MeATP by 34 ± 4 and 20 ± 3%, respectively, (data not shown, $n = 3$). Fig. 2B shows that ethanol inhibition of current activated by 10 μM ATP was concentration-dependent over the concentration range 2.5–500 mM. The calculated ethanol concentration that produced 50% inhibition (IC_{50}) was 83 ± 6.4 mM, the slope factor was 1.0 ± 0.1, and the maximal effect was 100% inhibition. Over the concentration range 2.5–500 mM, application of ethanol alone did not activate detectable current ($n = 4$).

To examine whether inhibition of ATP-gated channels by ethanol was voltage-dependent, the amplitude of current activated by 10 μM ATP was measured over a range of

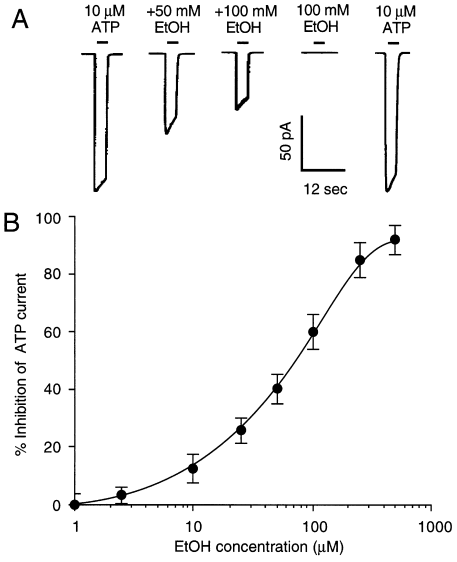

Fig. 2. Inhibition of ATP-activated current by ethanol. (A) Current activated by 10 μM ATP before, during, and after application of 50 and 100 mM ethanol (EtOH), and the application of 100 mM ethanol in the absence of ATP. Records are sequential current traces (from left to right) obtained from a single neuron. (B) Graph plotting average percentage inhibition of the amplitude of current activated by 10 μM ATP as a function of ethanol concentration (1, 2.5, 10, 25, 50, 100, 250 and 500 mM). Each point is the average of 4–8 cells. The calculated IC_{50} value for EtOH inhibition of ATP activated current was 83 ± 6.4 mM, the slope factor was 1.0 ± 0.1, and the maximal effect was 100% inhibition.

holding potentials in the absence and presence of 50 mM ethanol. As shown in Fig. 3, in the absence of ethanol current activated by 10 μM ATP exhibited inward rectification and an average reversal potential of -5 ± 4 mV ($n = 4$). Ethanol did not change the reversal potential of ATP-activated current (-3 ± 4 mV; Student's t-test, $P > 0.1$; $n = 4$), and the effect of ethanol did not differ significantly at membrane holding potentials between -80 and $+40$ mV (ANOVA, $P > 0.25$; $n = 4$-5; data not shown).

To examine whether the concentration of ATP affects the inhibition by ethanol, the magnitude of inhibition of ATP-activated current by ethanol was determined at various concentrations of ATP. As illustrated in Fig. 4A, the magnitude of ethanol inhibition was reduced by increasing the concentration of ATP from 5–100 μM. Concentration-response analysis (Fig. 4B) revealed that 100 mM ethanol shifted the ATP concentration-response curve to the right in a parallel manner, increasing the EC_{50} for ATP from 18 ± 0.7 μM in the absence of ethanol to 33 ± 0.4 μM in the presence of ethanol (ANOVA, $P < 0.05$), without altering the slope (1.4 ± 0.1 vs. a control value of 1.3 ± 0.1; $P > 0.05$) or maximal value of the ATP concentration-response curve ($P > 0.2$).

Extracellular ATP has previously been reported to activate inward current via activation of P2X receptors in cultured rat hippocampal neurons [1,9] and in CA1 neurons of rat hippocampal slice [12,19]. In the present study, we recorded ATP-activated inward current in a subset of adult rat hippocampal neurons freshly isolated from the CA1 region. Inward current was also activated by the P2 receptor agonists 2-MeSATP and α,β-MeATP, and inhibited by the P2 receptor antagonist PPADS. In addition, low micromolar concentrations of Zn^{2+} markedly potentiated the ATP-activated current. These pharmacological characteristics of ATP-activated current in our experiments are similar to those observed in cultured rat hippocampal neurons [1] and in the CA1 neurons of rat hippocampal slice [19], and

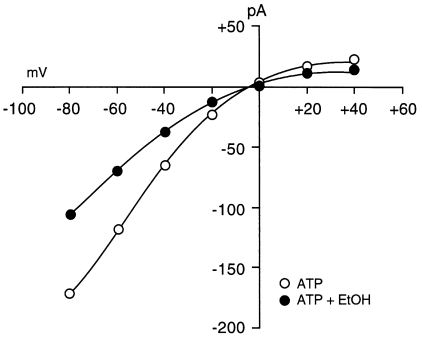

Fig. 3. Effect of membrane potential on inhibition of ATP-activated current by ethanol. Current-voltage relationship (I–V plot) showing the amplitude of current activated by 10 μM ATP as a function of membrane potential, in the absence and presence of 50 mM ethanol in a single neuron. Note that ethanol did not alter the reversal potential of ATP-activated current.

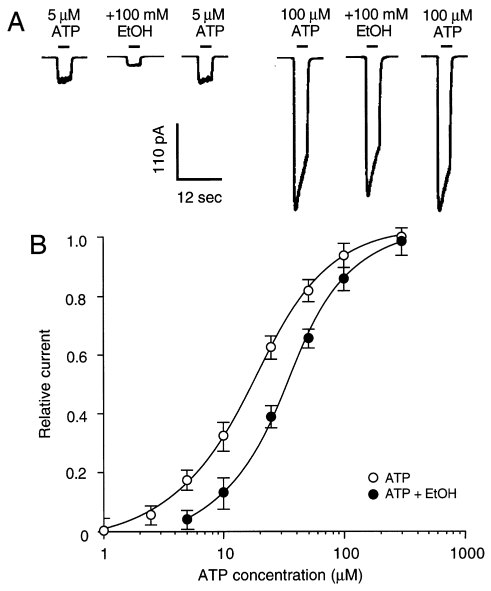

Fig. 4. Effect of ATP concentration on ethanol inhibition of ATP-activated current. (A) Records showing currents activated by 5 μM ATP (left traces) and 100 μM ATP (right traces), before, during, and after application of 100 mM ethanol in a single neuron. (B) Graph plotting the relative amplitude of ATP-activated current in the absence and the presence of 100 mM ethanol as a function of ATP concentration (1, 2.5, 5, 10, 25, 50, 100 and 300 μM). Amplitude is normalized to the current activated by 300 μM ATP in the absence of ethanol. Each data point is the average of 4–6 cells. Ethanol significantly increased the EC_{50} value for ATP from 18 ± 0.7 μM in the absence of ethanol to 33 ± 0.4 μM in the presence of 100 mM ethanol (ANOVA, $P < 0.01$).

are consistent with the involvement of P2X receptors. So far, the amino acid sequence, molecular structure, and subunit composition of P2X receptors in rat hippocampal neurons have not been identified. Although northern blot analysis and in situ hybridization studies have revealed strong expression of $P2X_4$ mRNA in rat hippocampus [3,20,22], a recent study indicated that homomeric $P2X_4$ receptors are not the primary subtype of P2X receptor in rat hippocampal CA1 neurons [12].

The results reported here also show that the inward current activated by ATP was inhibited by ethanol in a concentration-dependent manner with an IC_{50} value of 83 mM. In humans, this concentration of ethanol is associated with severe intoxication and loss of consciousness [15]. The observation that ethanol shifted the ATP concentration-response curve to the right in a parallel manner, increasing the EC_{50} value for ATP, is consistent with an action of ethanol to decrease the apparent affinity of the receptor for agonist. This observation agrees well with previous findings for ethanol inhibition of P2X receptors in bullfrog DRG neurons [14,23] and recombinant rat $P2X_4$ receptors expressed in *Xenopus* oocytes [27]. In addition, the voltage-independence of ethanol inhibition suggests that ethanol does not alter the ion permeance ratio of

ATP-gated channels, nor does it induce a conformational change in the channel protein that is voltage-dependent [16].

Ethanol has been reported to alter hippocampal function [24]. Recent evidence suggests that P2X receptors may play a role in the function of the mammalian hippocampus. First, several P2X receptor subunits, including $P2X_1$, $P2X_2$, $P2X_4$ and $P2X_6$, have been found to be localized in rat hippocampus [3,13,20,22]. Second, ATP can be released in a calcium-dependent fashion by electrical stimulation of presynaptic nerve fibers in rat hippocampal slice [25] and by glutamate-stimulation of cultured rat hippocampal neurons [10]. Third, excitatory postsynaptic currents mediated by ATP have been recorded in rat CA1 pyramidal neurons [19]. Fourth, exogenously applied ATP can activate inward ion current [1,9,12,19] and evoke an increase in the intracellular Ca^{2+} concentration in rat hippocampal neurons [10,19]. Fifth, ATP application can induce a long-lasting enhancement of population spikes recorded from mouse [26] and guinea pig [7,18] hippocampal slice. In view of these reports, our observation that ethanol can inhibit ATP-activated current in hippocampal neurons suggests that P2X receptors could be important effectors of ethanol action in the hippocampus.

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